MICROBIAL COMPOSITION AND CELL VIABILITY OF A MULTI-STRAIN BACTERIA FORMULATION

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Introduction

A probiotic formula to be functional and reliable should: i) be taxonomically defined; ii) contain viable cells, iii) have a reproducible composition, iv) and ideally, should be controlled for probiotic molecular markers. Here we detail the consistency of the multispecies probiotic product VSL#3, which has been produced for the last 20 years and is marketed globally for threating inflammatory bowel disease, pouchitis and other intestinal diseases.
To show consistency in the quality, viability and composition of the multispecies probiotic product VSL#3 various batches of the multispecies probiotic VSL#3 were analyzed in detail and derived from productions in the USA and Italy. The product batches have been tested using a series of microbiological, phylogenetic and metagenetics methods. The microbiological analysis included plating on selective media, cell counting and viability analysis by Flow Cytometry (FCM) using fluorescent dyes that allowed high throughput separation and quantification of live, dead and damaged cells. A metagenetic approach, based on *16S rRNA* gene profiling, was used to define the bacterial community structure of different productions batches. In addition, *Lactobacillus helveticus* and *Lactobacillus acidophilus* S-layer proteins, which are known to exert anti-inflammatory effects by reducing the activation of NF-⊠B on the intestinal epithelial Caco-2 cell line, have been extracted, visualized on SDS-PAGE and identified by nLC-ESI-MS/MS analysis. Moreover, urease activity of *Streptococcus thermophilus*, known to exert positive effect on human health by competing with the undesired urease-positive bacteria of the human microbiota, was quantified using a spectrophotometric-, and flow cytometry-based assay.

Results and discussion

Taxonomy and viability of the multi-strain probiotic formulation
The different batches tested were all found to contain a common bacterial community structure based on the presence of the following species *Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus helveticus, Bifidobacterium breve* and *Bifidobacterium animalis* subsp*. lactis.* The stability of the batches was confirmed by FCM, and viable cells were always above the value of 2 x 1010 event/g. FCM analysis allowed to identify and quantify live, dead and damaged cell populations in the multi-strain probiotic formulation (Figure 1) without any information on the species distribution within each of those populations. However, it is quite relevant for the probiotic fate of the product, be able to address which is the viability of each species within a multi-strain formulation. This goal was achieved through the quantification of the relative abundance of each probiotic species using species-specific-qPCR assay performed on total DNA extracted from live and dead cell populations separated by FACS. Interestingly, the data obtained revealed that the species in the multi-strain formulation showed a species-specific viability level (Figure 1).

Putative probiotic molecular markers
The *L. helveticus* and *L. acidophilus* S-layer protein SlpA were detected in each VSL#3 batches tested, representing the majority of the surface proteins with a molecular weight ranging between 40 and 50 kDa, thus highlighting that this relevant immunomodulatory factors were not subjected to degradation during the preparation and the shelf-life of the multi-strain probiotic formulation. Likewise, urease activity peculiar of the species S. *thermophilus* was stable in all VSL#3 batches tested.

Conclusions

In conclusion, stability, molecular and taxonomic comparative analysis show that VSL#3 is reliably and reproducibly produced in different parts of the world. More importantly, the assessment and the quantification of putative probiotic molecular markers (S-layer proteins and urease) directly in the product, *i. e.* without a cultivation step, represents a first example of quality control in a probiotic product targeted to “probiotic-traits”, when quality controls are currently directed and limited to the evaluation of cell viability. In addition, the quantification of the viability by FCM combined with a cell sorting and a qPCR quantification of each bacterial species in the blend, represent a further new quality control step for a multi-strain probiotic product.

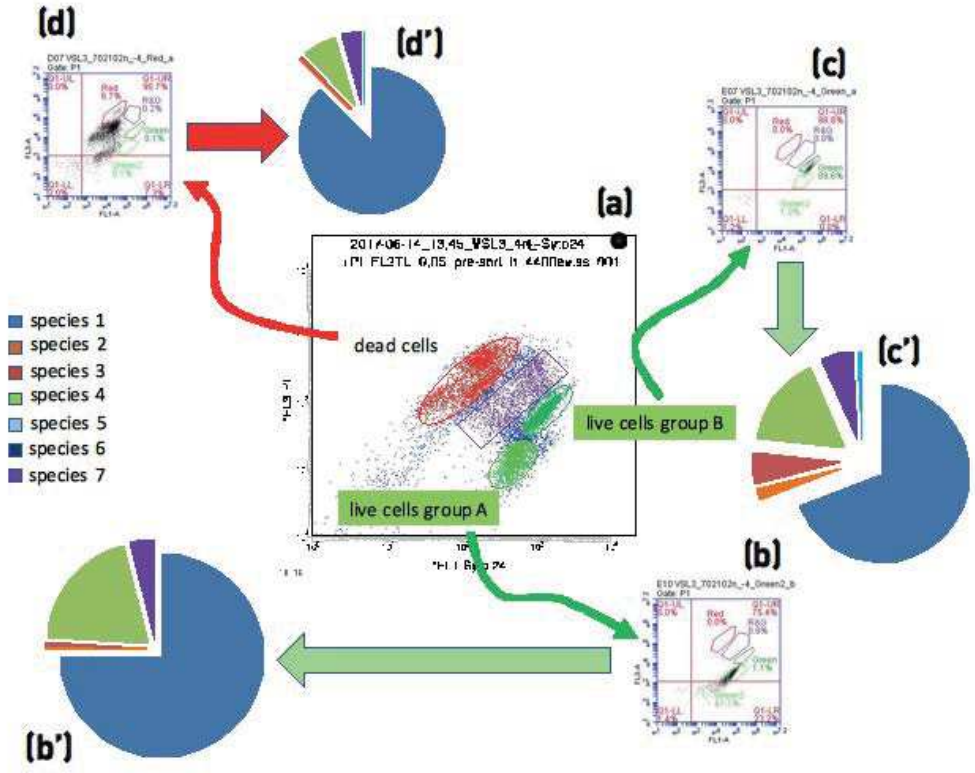

Figure 1. Quantification of live and dead cell population in the multi-strain probiotic formulation by FCM according to ISO19344-IDF232 (a). Post-sorting analysis by FCM of live and dead cell populations (b), (c) and (d). qPCR-based quantification of the relative abundance of the microbial species in the multi-strain probiotic formulation (b'), (c') and (d').

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